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The long 3′-untranslated regions of the PDGF-A and -B mRNAs are only distantly related

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A cDNA clone of about 2300 base pairs was prepared from the human osteosarcoma cell line U-2 OS by hybridization with a 22-mer oligonucleotide complementary to the NH_2-terminus of PDGF-A. Restriction and sequence analysis showed that this clone contains the entire coding region for PDGF-A and a long 3′-untranslated region which is only distantly related to that in the mRNA of PDGF-B.

Platelet-derived growth factor; cDNA; Oncogene; (Tumor cell)

1. INTRODUCTION

Human platelet-derived growth factor (PDGF) is composed of dimers of homologous polypeptide chains, termed A and B [1–3]. It is unknown, whether human PDGF is a heterodimer or a mixture of homodimers, but it has been shown that homodimers of both types (A-A or B-B) express the full biological activity [4–6].

The PDGF-B gene (c-cis) has been mapped on the long arm of chromosome 22, the PDGF-A gene on chromosome 7 [7]. The different location of the two genes has suggested a different regulation of the transcription. Indeed in numerous tumor cells the two genes are expressed to quite a different extent [7–9]. Interestingly enough only those cells that express the PDGF-A gene secrete a PDGF-like mitogen into the medium which has been identified as PDGF-A homodimer. There is some evidence that untranslated 3′-regions might have some function in expression and/or stability of the mRNA [10–12]. To investigate a possible role of the unusually long 3′-moieties of the PDGF mRNAs we have cloned and sequenced the mRNA of PDGF-A.

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2. MATERIALS AND METHODS

2.1. *Materials*

Restriction endonucleases, T_4 ligase, T_4 DNA polymerase, DNA polymerase I and the large fragment of DNA polymerase I (Klenow fragment) were purchased from Boehringer. Sequenase was from USB. Nucleotides were from Pharmacia Biochemicals. Antibiotics were purchased from Sigma. Radioactive nucleotides were from Amersham. *E. coli* strains 5K or JM103 were used for transformation by pBR322 or M13 vectors, respectively.

2.2. *Cell culture*

Osteosarcoma cells (U-2 OS) were kindly provided by Dr B. Westermark (Uppsala, Sweden) and were grown as described [13].

2.3. *Construction and screening of a cDNA library*

The same cDNA library was used that was constructed for the isolation of the PDGF-B gene [14]. This library was screened by standard techniques [15] using a synthetic 22-mer oligonucleotide complementary to the amino-terminus of the PDGF-A chain.

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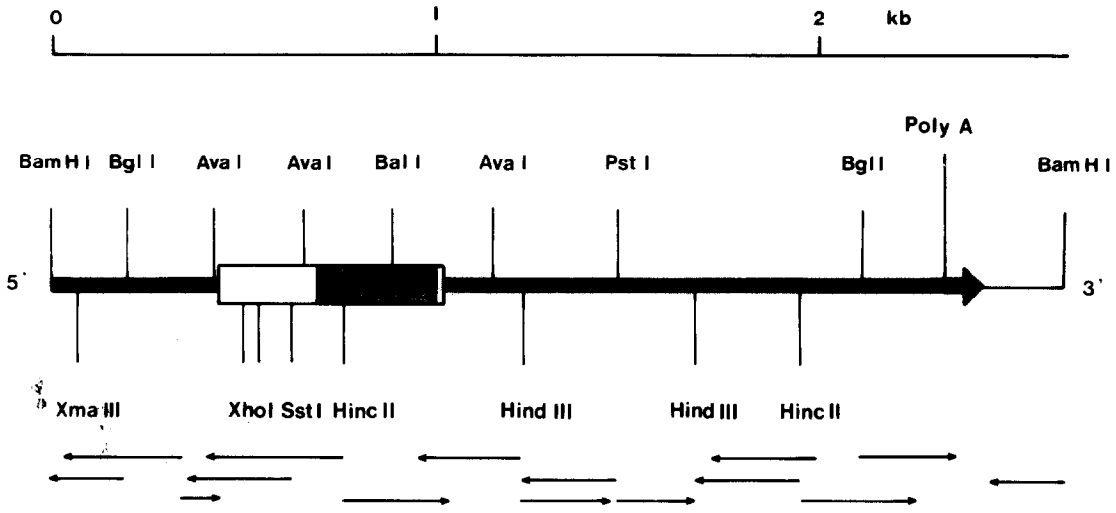

Fig.1. Restriction map of the PDGF-A cDNA clone pPGF-2. The thick arrow represents the cDNA insert in pPGF-2. The insert is flanked on the left and right by restriction sites from the vector pBR322. The PDGF-A coding region is indicated by boxes. The sequence coding for mature PDGF-A is shaded. The small arrows indicate the sequenced fragments.

2.4. *DNA sequencing*

Restriction fragments from clones pPGF-1 and pPGF-2 were subcloned in M13 mp 18 or M13 mp 19. In one case a fragment was obtained by shortening the 1 kb *Bam*HI/*Hin*dIII fragment with exonuclease III [16]. Single-stranded DNA was sequenced using the method of Sanger following the protocol of the sequenase system described by the manufactures (USB).

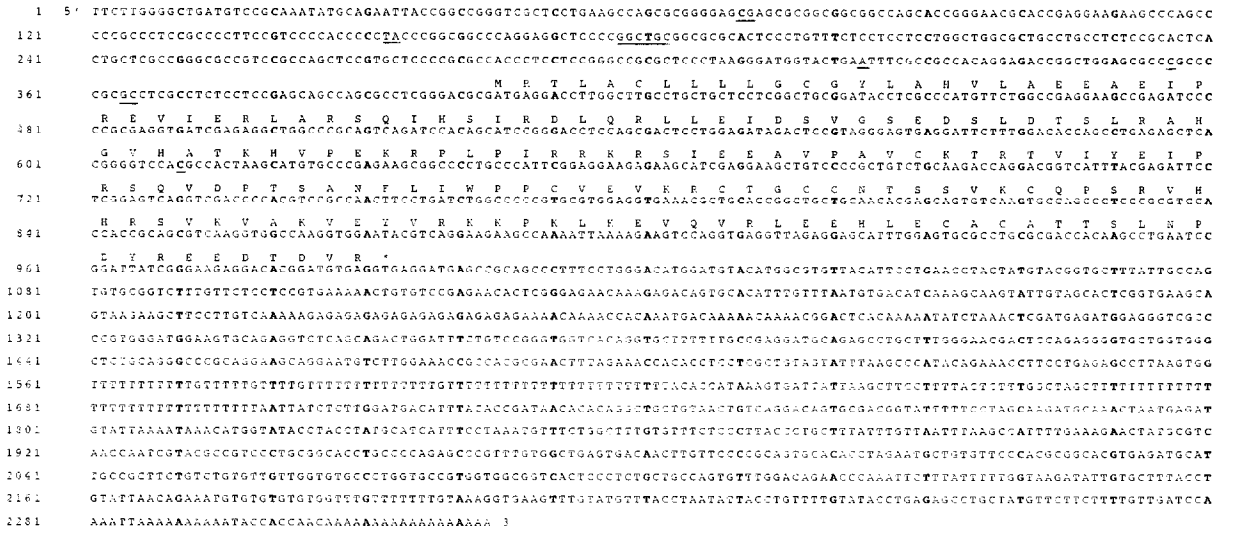

Fig.2. Nucleotide sequence of the cDNA insert of clone pPGF-2 and deduced amino acid sequence of PDGF-A. Alterations in the DNA sequence from that of D1 are underlined.

3. RESULTS

Screening of 80 000 colonies of the U-2 OS cDNA library with a synthetic 22-mer oligonucleotide primer resulted in the isolation of two plasmids pPGF-1 and pPGF-2 which contained inserts coding for PDGF-A. pPGF-1 is regarding its length of 1.2 kb very similar to clone D1 [7] and contains the entire coding region for the prepro PDGF-A (not shown). Plasmid pPGF-2 has an insert of ≈ 2.3 kb. Restriction analysis (fig.1) revealed that the additional 1000 bases are located at the 3′-end of the coding region. Thus like in the PDGF-B mRNA there is a long untranslated 3′-region.

Fig.2 shows the DNA-sequence of the insert of plasmid pPGF-2. The sequence extents 15 bases further at the 5′-end compared to that of the reported clone D1 [7]. Some alterations have been found that may reflect small differences between the two cell lines from which the cDNA clones were isolated. Both clones (pPGF-1 and pPGF-2) exhibit a deletion of 69 bases starting from position 984, predicting an A chain precursor 15 residues smaller and lacking the basic C-terminal region. The $d(GA)_n$ stretch behind the coding region was considerably longer [7]. The following A-rich region differed markedly from that in clone D1.

Two extremely T-rich regions are located between position 1560 and 1700. The first stretch comprises about 60 T-residues interrupted by four G-residues. The second one contains 31 uninterrupted T residues. The appearance of the polyadenylation consensus sequence ATTAAA at the 3′-end indicates that the entire 3′-portion has been cloned.

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A)  AGAGAGAGAGAGAGA                PDGF-A
    AGAGTGTGAGAGAGA                PDGF-B

          ↓     ↓     ↓

B)  TTTTTTTTGTTTTTGTTTTG           PDGF-A
    TTCTTTTCGTTTTCGTTTTG            PDGF-B

C)  TTTGTTTTTT     GTAAA            PDGF-A
    TTTATTTTTTAAATGTAAAA            PDGF-B
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Fig.3. Possible homologous region in the mRNAs of PDGF-A and PDGF-B. (A) region 1, ≈ 250 bases from the end of coding region; (B) region 2, ≈ 600 bases from the end of the coding region; (C) region 3, ≈ 80 bases from the 3′-end.

4. DISCUSSION

Multiple PDGF-A mRNA transcripts in the range of 2.8–1.7 kb are visualized after Northern Blot analysis of several cell lines [7–9]. Also, the splicing pattern differs between the investigated cell lines [8] as indicated by the different intensities of the bands. In human cells the most abundant species exhibit a size of 2.3 kb. This length agrees well with the cDNA insert of 2.3 kb found in clone pPGF-2.

The most intriguing features of the PDGF-A mRNA sequence are the extremely long T-stretches in the untranslated 3′-region. Especially notable is the uninterrupted stretch of 31 T residues starting from position 1668 (fig.2). This sequence might form a stable secondary structure with the poly(A) tail preventing the first strand synthesis from the 3′-end. Thus priming might occur internally from the A-rich region shortly behind the translated region as observed previously [7] and with clone pPGF-1.

PDGF-A and PDGF-B are regulated differently in a variety of tumor cells. Very recent findings have shown, that the 3′-noncoding sequences may play an important role in controlling gene expression [12]. We therefore compared the sequences of the untranslated 3′-regions of the PDGF mRNAs. There are, if any, only distantly related sequences in the two mRNA species (fig.3). The first region (≈ 250 to 300 nucleotides 3′ from the coding region) comprises the repeat of d(GA). The second homologous region (≈ 600 nucleotides from the coding region) contains the first T-rich region in the PDGF-A sequence. The similar spacing of the G residues in both sequences might indicate a homology and a common function.

It has been discussed that certain sequences is proximity to the 3′-end of a mRNA are conserved amongst different proteins including PDGF-B, nerve growth factor, interferon-β, and

interleukin-2 [16]. Such sequence are largely absent in the PDGF-A mRNA. Possibly a homologous sequence ≈ 80 residues from the 3′-end may be present present (fig.3). Furthermore, the two mRNA species differ markedly in their A-T content in the 3′-proximal region. Based on the highly conserved amino acid sequence of the mature PDGF-A and PDGF-B proteins one has to assume that the two forms originate from a gene duplication. Interestingly, the A- and B-chain gene have acquired different chromosomal localizations and considerable alterations in the noncoding regions have occurred, which might have some influence on the stability of the mRNAs and/or on the regulation of transcription. It remains to be determined if these alteration have any significance regarding regulation and biological properties of the two PDGF species.

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REFERENCES

[1] Johnsson, A., Heldin, C.-H., Westermark, B. and Wasteson, A. (1982) Biochem. Biophys. Res. Commun. 104, 66–74.
[2] Doolittle, R.F., Hunkapiller, M.W., Hood, L.E., Devare, S.G., Aaronson, S.A., Robbins, K.C. and Antoniades, H.N. (1983) Science 221, 275–276.
[3] Waterfield, M.D., Scrace, G.T., Whittle, N., Stroobant, P., Johnsson, A., Wasteson, A., Westermark, B., Heldin, C.-H., Huang, J.S. and Deuel, T.F. (1983) Nature 304, 35–39.
[4] Stroobant, P. and Waterfield, M.D. (1984) EMBO J. 3, 2963–2967.
[5] Kelly, J.D., Raines, E.W., Ross, R. and Murray, M.J. (1985) EMBO J. 4, 3399–3405.
[6] Heldin, C.-H., Johnsson, A., Wennergren, S., Wernstedt, C., Betsholtz, C. and Westermark, B. (1986) Nature 319, 511–514.
[7] Betsholtz, C., Johnsson, A., Heldin, C.-H., Westermark, B., Lind, P., Urdea, M.S., Eddy, R., Shows, T.B., Philpott, K., Mellor, P.L., Knott, T.J. and Scott, J. (1986) Nature 320, 695–699.
[8] Sejersen, T., Betsholtz, C., Sjölund, M., Heldin, C.H., Westermark, B. and Thyberg, J. (1986) Proc. Natl. Acad. Sci. USA 83, 6844–6848.
[9] Weich, H.A., Herbst, D., Schairer, H.U. and Hoppe, J. (1987) FEBS Lett. 213, 89–94.
[10] Yaffe, D., Nudel, U., Mayer, Y. and Neuman, S. (1985) Nucleic Acids Res. 13, 3723–3737.
[11] Treisman, R. (1985) Cell 42, 889–902.
[12] Owen, D. and Kühn, L.C. (1987) EMBO J. 6, 1287–1293.
[13] Betsholtz, C., Westermark, B., Ek, B. and Heldin, C.H. (1984) Cell 39, 447–457.
[14] Weich, H.A., Sebald, W., Schairer, H.U. and Hoppe, J. (1986) FEBS Lett. 198, 344–348.
[15] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[16] Henikoff, S. (1984) Gene 28, 351–359.